site-directed mutagenesis studies in combination with patch-clamp experiments. Our results suggest that the efficacy of the agonists is closely coupled to the energetics of the C-loop of the receptor. With the presence of different ligands in the binding sites, the C-loop closure energy profiles given by potential of mean force (PMF) calculation reveal interesting differences. The results suggest that the combination of modelling and patch-clamp experiments for partial agonists can be a powerful approach to deciphering the atomistic details of glycine receptor activation.

2771-Pos Board B463
Towards Structural and Functional Determination of a Non-Desensitizing α1 Glycine Receptor
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Glycine receptors (GlyR's) are inhibitory ligand-gated receptors in the nicotinicoid receptor superfamily. GlyR's mediate neurotransmission in CNS and are typically activated by glycine. GlyR is implicated in pain signaling to the brain. In order to better understand the silencing electrical activity of the brain and also the structure and function of GlyR in its open state, ivermectin (IVM) sensitive GlyR channels are developed as IVM is shown to stabilize GlyR in its non-desensitizing state. Double mutant F207A/A288G in α1 human GlyR has been shown to increase IVM sensitivity and reduce/remove sensitivity for glycine. We are developing photo crosslinking methodologies linked with mass spectrometric analysis on systematically generated single Cys mutations in IVM sensitive GlyR to enable us to study state-dependent structure of GlyR in the open state. Studies on GlyR in its open state will provide distance constraints that can be used in computational models to better the structure in its open state (non-desensitizing) and can help perform comparative studies with a desensitizing GlyR.

2772-Pos Board B464
Methods for Identification of State-Dependent Crosslinks for Structural Determination of Membrane Proteins
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Determining the structure of membrane proteins is critical to understanding how they function. Though various techniques exist to elucidate structural information, often times they lack the ability to determine allosteric movements associated with the dynamic nature of the proteins. Development of a method that affords the possibility of mapping changes in structure associated with the complicated allosteric mechanisms of membrane proteins is a valuable tool to better understanding the structural basis of protein function. The glycine receptor (GlyR) is a ligand-gated ion channel associated with inhibition of signal propagation in the central nervous system. Single-Cys mutant homomeric human α1 GlyR is overexpressed in insect cells, purified, and reacted with a methanethiosulfonate-benzophenone heterobifunctional crosslinker containing an alkyne tag. After state-dependent photoactivation in the presence or absence of modulatory ligands, inter- and intra-subunit crosslinks may be isolated by proteolysis, reduction, click chemistry and affinity chromatography. Verification and quantitation of crosslinked species may be sensitively identified by fluorescent tagging and use of a HPLC-microfluidic-laser induced fluorescence (LIF) system. Structural information can be derived through the implementation of multi-dimensional mass spectrometry to identify sites of crosslinking and relative distances which can be used to refine computational models in state specific manner. Development of this method, using GlyR as a model system, will allow for structural determinations to be made on any protein and thus allow for a better understanding of the dynamic nature of allosteric proteins.

Ion Channels and Disease I

2773-Pos Board B465
Learning the Kinetics of Amyloid β Pore in Alzheimer's Disease Pathology
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Increased synthesis of self-aggregating amyloid beta (Aβ) peptides caused by abnormal processing of amyloid precursor protein (APP) is a hallmark of Alzheimer's disease (AD) pathogenesis. Aβ mediates it's effect by disrupting the integrity of cell membrane and interacting with plasma membrane channels. The soluble form of Aβ aggregates into calcium (Ca^{2+}) - permeable pores in the membrane. Aβ pores promote uncontrolled increase in the cytoplasmic Ca^{2+} concentration by allowing Ca^{2+} influx to the cell, in addition to enhancing the activity of Ca^{2+}- permeable channels on the plasma membrane and intracellular compartments. The Ca^{2+} influx through Aβ pores upsets the otherwise fine-tuned micrometer-sized elementary Ca^{2+} release events and whole-cell Ca^{2+} response. The disrupted Ca^{2+} signaling in turn has the potential to alter cell function in many ways.
Here, we have used computational modeling in conjunction with TIRF microscopy to study the function of Aβ pores in AD cells. TIRF microscopy was used to image Ca^{2+} flux through thousands of Aβ pores in parallel at the millisecond scale and single channel resolution. The fluorescence time-series from individual pores was idealized by extending the Maximum Likelihood-based method developed for separating signal from baseline in noisy quantal data (Bruno et al. 2013, Biophys. J. 105:68). The idealized data was used to developed data-driven models for the kinetics of Aβ pore at different stages of it's life. In addition to providing deep insights into the kinetics of Aβ pore, this study demonstrates that the massive imaging data obtained from thousands of channels in parallel using TIRF microscopy can be utilized for single molecule modeling in the same manner as electrical patch-clamp data. Employing the optical patch-clamp data for Markov chain modeling has the added advantage of the experiments being done under physiological conditions.

2774-Pos Board B466
Investigating How Aβ and αSynuclein Oligomers Initially Demage Neuronal Cells
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Scanning Ion Conductance Microscopy (SICM) allows simultaneous measurements of various important structural and functional parameters with nanometre resolution on living cells. In addition, the pipette allows defined dosing of substances to cells. Amyloid beta 42 is a well known component of extracellular amyloid plaques in association with Alzheimer's disease. Similarly, alpha synuclein is the main component of intracellular Lewy bodies in Parkinson's disease. The initial mechanism by which these proteins cause cellular cytotoxicity remains elusive. Solutions containing oligomers of these bio molecules were locally delivered, via the SICM pipette, to the surface of neurons and glial cells in a quantitative fashion. The resulting Ca^{2+}-influx was monitored over a 10 min period and taken via an EMCCD camera. These experiments provide new insights into the molecular mechanism by which protein oligomers initially damage cells and how many oligomers are required.

2775-Pos Board B467
Acute Effects of β-Amyloid (1-42) Oligomers on Rat Pyramidal Entorhinal Neurons
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Deposition of beta-amyloid peptide (Aβ) in senile plaques is the hallmark of Alzheimer disease neuropathology. Soluble Aβ oligomers block voltage-gated ion channels as P/Q type calcium channels (Nimmrich et al., 2008) as well as the transient potassium current (Liudyno et al., 2012) and calcium dependent potassium channels, BK type (Yamamoto et al., 2011). Exposure to Aβ may cause a loss of cellular calcium homeostasis, but the mechanism by which this occurs is uncertain. In this work we evaluated the acute response of rat pyramidal entorhinal neurons to oligomers formed from purified Aβ1-42 in both entorhinal cortex slice preparations and isolated pyramidal neurons in culture using current- and voltage-clamp conditions. Exposure to Aβ oligomers but not monomers increased the input resistance and enlarged the action potential in the slice preparation, whereas in culture neurons produced a reversible inhibition of the inward potassium current generated by voltage ramps from −70 to 70 mV in symmetric potassium conditions. This current is generated by inward rectifier potassium channels as well as the leak potassium channels, it was blocked by barium, arachidonic acid, bupivacaine and extracellular pH acidification, suggesting that TASK type K2P channels are targets of the toxic effects of Aβ. Blockage of potassium channels by Aβ could lead to prolonged cell depolarization, thereby increasing calcium influx.
Supported by CONACyT (324341) Mexico.

2776-Pos Board B468
Disregulation of Calcium Homeostasis Connected with Familial Alzheimer's Disease
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Familial Alzheimer's disease (FAD) caused by mutations in presenilin-1 (PS1) gene in approximately 50% of cases and in Amyloid precursor protein (APP)